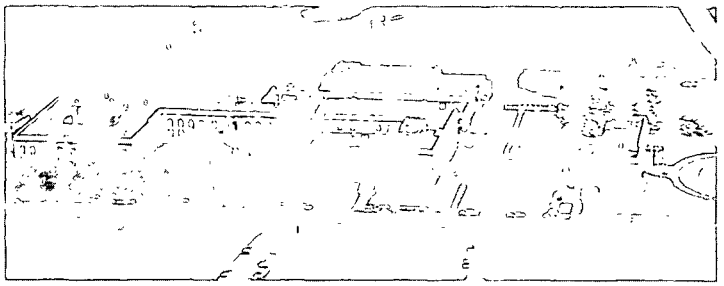

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THE PEROXYACETIC ACID DELIGNIFICATION OF WHITE BIRCH AND NEW EVIDENCE FOR LIGNIN-CARBOHYDRATE BONDS

A. J. GLINSKI AND G. A. NICHOLLS

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FORWARD

Peroxyacetic acid is one of the nonsulfur chemicals that can delignify wood by oxidation. Since this reaction takes place selectively without significant degradation of the cellulose, peroxyacetic acid is of considerable potential interest for environmental and yield reasons.

The mechanisms of peroxyacetic acid delignification of wood is very complex and is known to proceed with loss of carbohydrate material, particularly hemicellulose. At one time it was believed delignification was a random process and the main differences in the degradation products were physical rather than chemical. As more sophisticated methods have been applied to this complex problem this concept has changed.

The study described here shows that peroxyacetic acid delignification proceeds with some degree of order, which is seen from chemical differences of the products. These products have been found to have carbohydrates bonded to the phenylpropane units of lignin. This was determined using optical measurements like those applied in the study of other naturally occurring polymers, such as proteins and polypeptides. It is believed this is the first report where these techniques have been successfully applied in lignin chemistry.

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The Peroxyacetic Acid Delignification of White Birch and New Evidence for Lignin-Carbohydrate Bonds

A. J. Glinski and G. A. Nicholls*

SUMMARY

Stepwise delignification of white birch with peroxyacetic acid was followed by fractionation of the solubilized material on Porasil B to give about half as powders by freeze-drying fractions between the void volume, $\underline{V}_o$, and the total liquid volume, $\underline{V}_t$. The size range of the solubilized molecules was unchanged as delignification proceeded but there were more of the largest molecules. Carboxyl and methoxyl contents of the freeze-dried materials were 0.5-0.8 and 0.2-0.6 per C_9 unit, respectively, and absorptivities were 0.8-11.3 liters/g cm. These and infrared data, which had some similarity to absorptivities as delignification proceeded, were indicative of significant aromatic ring degradation. There was an increase in polysaccharide content of the freeze-dried materials as delignification proceeded and an essentially analogous increase in the xylan component including infrared spectral evidence for an increase in glucuronoxylan content. Electrophoretic and enzymatic experiments provided no indication that the solubilized lignin was separable from the carbohydrate. As delignification and fractionation proceeded there was an ordered pattern to the specific rotation of the freeze-dried materials, and this pattern was related to xylan, glucan and galactan content of the polysaccharides. ORD and CD curves showed that a positive Cotton effect in the early fractions near the start of the delignification changed to a negative Cotton effect in late fractions near the end of the delignification. This is believed to occur because hydrodynamic size is related to the composition and specific rotation of the carbohydrate moiety in such a way that there is significant ordering of molecular structures capable of giving Cotton effects, and it is concluded that the carbohydrates are bonded to the phenylpropane units of lignin.

*A. J. Glinski, Graduate Student and G. A. Nicholls, Professor of Pulp and Paper Technology, The Institute of Paper Chemistry, Appleton, Wisconsin 54911, U.S.A.

INTRODUCTION

In a previous paper, the fractionation and examination of soluble products from oxidative delignification of loblolly pine by peroxyacetic acid was described (1), then what happens to molecular size distribution and polysaccharide content as delignification proceeds was reported (2). This is a somewhat comparable study on white birch.

RESULTS AND DISCUSSION

Delignification

Acetone extracted Betula papyrifera or white birch wafers were reacted with peroxyacetic acid in a continuous flow apparatus as described previously (1), except that reaction was at about 50°C, as indicated in Table I. This shows that with 71% lignin removal the apparent material unaccounted for was 3.3% and from sugar analyses, by difference, this material loss would appear to be mainly polysaccharides. Such losses during the peroxyacetic acid delignification of hardwoods are consistent with other work (4).

[Table I here]

To determine whether reaction was uniform across the wafers, stained cross sections were microscopically examined. No indication of nonuniform reaction was observed and at 98% yield with phloroglucinol stain there was a noticeable loss of the usual red tone suggesting early attack on lignin monomer units containing α,β-unsaturated carbonyl groups in the phenylpropane side-chain. This is comparable to previous observations on loblolly pine (1,5).

The liquors containing lignin solubilized by peroxyacetic acid were collected for each reaction run either as a composite or as six aliquots (fractions of liquor) from consecutive times of reaction as in Table II.

[Table II here]

Fractionation on Porasil B

Since fractionation as in gel filtration is influenced by sample concentration or volume (6), liquors as above were evaporated under reduced pressure to a standard dissolved solids content of about 12 g/l. Porasil B was chosen as the column packing on the basis of previous work (1,2) and preliminary tests. Fractionations of composite liquors and aliquots of liquor were carried out using the same apparatus and conditions as for loblolly pine (1,2).

Each fractionation on Porasil B was monitored by absorbance at 280 nm, which corresponds to a UV spectrum maximum and is plotted on a normalized basis vs. fraction number and volume of eluate in Fig. 1, for the six liquor aliquots as in Table II. The curves have a general similarity to the corresponding curves obtained for loblolly pine (2). These also were without any sharp peak immediately following the void volume of eluate V_o (1,270 ml) and had significant absorbance after the total liquid volume, V_t (2,550 ml) (1), the interpretation of which has been discussed previously (2). In a separate but similar fractionation of a composite liquor a sample of Fraction 45 was used to confirm the applicability of Beer's Law.

[Fig. 1 here]

As was found previously for loblolly pine (1), the materials from Fractions 1-65 could be obtained as fluffy powders by freeze-drying, whereas the remaining fractions gave sticky gums. Further experiments were focused on these fluffy powders.

It is possible that there is ionic or polar interaction between Porasil B and solute during fractionation as in Fig. 1. To test this, Fractions 1-65 from the third and sixth liquor aliquots were combined and fractionated again before and after methylation to give the curves in Fig. 2. Potentiometric titrations showed that methylation reduced the carboxyl content of the solubilized lignin before fractionation from 14.5 to 0.6%. Thus, the considerable similarity of the curves in

Fig. 2 greatly reduces the likelihood of there being any significant ionic or polar interaction between the column packing and solute.

[Fig. 2 here]

Also, in Fig. 1 the progressive increase in height of the first part of each curve (particularly up to about Fraction 40) as delignification proceeded is interpreted as an increase in the amount of the largest molecules eluted as delignification proceeded. A similar result was obtained for loblolly pine (2).

For further investigation Fractions 1-65 from each of the six aliquots (Fig. 1) was converted into twenty groups to give a total of 120. The combining of fractions into a group was based on the number of consecutive fractions needed to give about 5 w/w% of the freeze-dried material when fractionating a composite liquor.

Changes in carboxyl, methoxyl and aromatic content

Carboxyl analyses on Fractions 12-15, 28-30, and 52-54 from the second and fifth aliquots of liquor showed 0.49-0.79 carboxyl per C_9 lignin unit. This is slightly higher than found for loblolly pine (1) and significantly higher than the 0.20 carboxyl per C_9 believed by others (7) to represent a minimum amount of degradation needed to achieve water solubility. The role that uronic acids might have in this situation is not clear.

There was also a significant reduction to 0.21-0.56 methoxyl per C_9 lignin unit compared with the more usual value of 1.2-1.6 methoxyls per C_9 unit in hardwood lignins (8). This reduction is about comparable to that observed for loblolly pine (1) and for birch dioxane lignin solubilized by peroxyacetic acid (7).

The NMR spectra were characterized by having very broad signals, for example, at about δ=7.0-6.3 and 4.0-3.5 for aromatic and aryl methoxyl protons, respectively (9). Deuterium exchange was used to show variation in the position of the signal for hydroxyl protons which tended to overlap the signal for aromatic

protons. Endeavors to move the hydroxyl proton signal out of the aromatic region were only partially successful since complete deuteration was not achieved. Thus this approach to a measure of the aromatic content of various fractions was unsuccessful.

On the basis of earlier work on loblolly pine, it was anticipated that absorptivities of the above groups of fractions might vary significantly. Measures of such variation as delignification and fractionation proceed are presented in Fig. 3. In this figure the first, third, and sixth groups of fractions, namely, Fractions 1-7, 28-30, and 58-61, have absorptivity ranges of 0.8-3.5, 5.3-8.4, and 7.1-11.3 liters/g cm, respectively, as delignification proceeded. The overall range of absorptivities, namely, 0.8-11.3 liters/g cm, brackets the range found for softwood lignin degraded by peroxyacetic acid (1). Compared with absorptivities at 280 nm of 12.1 and 13.1 liters/g cm reported for birch milled wood lignin and dioxane lignins (10,11), respectively, a significant decrease in most of the absorptivities is apparent in Fig. 3. Conceivably, these decreased absorptivities could arise from high polysaccharide content, but it appears that they tend to reflect ring degradation, as discussed below.

[Fig. 3 here]

In general, infrared spectra of the freeze-dried products from groups of fractions, as typified in Fig. 4, were resolved into fewer bands than usually observed in lignin (12). More specifically curves A, B and C in Fig. 4 illustrate, for the case of Fractions 12-15, spectral changes observed as delignification proceeded. Curve B' is for Fractions 62-65 from the same liquor aliquot as curve B and illustrates the smaller changes observed in the infrared spectra as fractionation proceeded.

[Fig. 4 here]

Compared with lignin, the spectra in Fig. 4 all show much greater absorption around 1730 cm^{-1}, which is in the carbonyl region. The absorption maximum in this region was no longer observable in the spectrum of the sodium salt for Fractions 12-15 from the second liquor aliquot. Instead, two strong absorption bands appeared at 1590 and 1400-1500 cm^{-1}, typical of carboxylate ions (13).

In Fig. 4 the bands near 1510 cm^{-1} which are characteristic of aromatic ring stretching vibrations and generally observed in lignins (12), indicate aromatic content was relatively greater after the initial stage of delignification. Also, as delignification proceeded there were other changes in absorption, particularly the appearance of distinct bands around 1370 and 1240 cm^{-1} which are characteristic of O-acetyl-4-O-methylglucuronoxylan from white birch and are believed to relate to CH bonding and C-O of acetyl, respectively (14). This indication of an increase in the relative amount of glucuronoxylan as delignfication proceeded is further supported by data on polysaccharide composition presented later.

A semiquantitative comparison of the aromatic content of groups of fractions was made on the basis of the 1510 cm^{-1} bands, applying a transmittance difference method (15) to partial spectra with an expanded transmittance scale. Confidence in this method is reduced when there is adjacent strong absorption and, therefore, the results in Fig. 5 are presented with some reservations. Nevertheless, there is a notable similarity in the ΔT 1510 cm^{-1} and absorptivity (Fig. 3) trends as fractionation proceeded. This situation makes it more likely that notable differences in chemical structure appear as fractionation proceeded. There is also some similarity in the same trends as delignification proceeded, but this is not as marked.

[Fig. 5 here]

Changes in polysaccharide content

Total polysaccharides found on a freeze-dried solids basis are shown in Fig. 6. This readily shows that the major trend was an increase in total polysaccharides in all fractions as delignification proceeded. This trend is different from those observed for absorptivity and infrared ΔT 1510 cm^{-1} data in Fig. 3 and 5, respectively, where particularly in the case of absorptivity, the major trend was significant change as fractionation proceeded. Thus, variation in total polysaccharide content is not apparently the major factor relating to variation in absorptivity.

[Fig. 6 here]

The relative amounts of polysaccharides calculated as xylan, glucan, galactan, arabinan, mannan and rhamnan found for different fractions ex-Porasil B as delignification proceeded are shown in Table III on a normalized basis. As for loblolly pine (2), there are orderly trends in the relative amounts of these components. For example, as delignification proceeded there was an increase in the relative amount of xylan found in all fractions, which ties in with the above noted appearance of bands around 1370 to 1240 cm^{-1} characteristic of O-acetyl-4-O-methylglucuronoxylan.

[Table III here]

Thus there are some notable changes in composition of the polysaccharides solubilized as delignification proceeded. Changes of composition as fractionation proceeded are less notable. Most of the major trend observed in Fig. 6 is accounted for by the xylan component on the basis of the data in Table III.

Although the total polysaccharide content of the freeze-dried fractions is only 5-25% it was pointed out earlier (2) that if some or all of the hemicelluloses are bonded to the degraded lignin, their influence on hydrodynamic size

might be greater than that of the attached lignin. The following part of this paper focusses on whether or not the lignin is bonded to the carbohydrate present in the various fractions.

Electrophoretic and enzymatic experiments

Although lignin-carbohydrate bonds and complexes have been the subject of much study and review (16,17), evidence for actual lignin-carbohydrate bonds is inductive rather than rigorous. For example, material containing lignin and xylan isolated from white birch has been studied previously (18). This included electrophoresis experiments in which the lignin and carbohydrate components did not separate, except after mild acid hydrolysis to break acid-labile bonds.

In the present study, electrophoresis was used to determine whether the solubilized lignin and carbohydrate in fractions ex-Porasil B could be separated. In essence Lindgren's procedure (19) was followed. As a check on technique, two solutions containing Fractions 45-50 from a composite liquor and either sapote gum or elm xylan were run. The electrophoretic mobility of the elm xylan was only slightly less than for Fractions 45-50 and the mixture of these two gave an elongated spot. The other mixture gave a clean separation. It was found, as might be anticipated simply on the basis of molecular size, that Fractions 5-9, 25-29 and 45-50 from a composite liquor had progressively greater electrophoretic mobility. Although these differences in mobility were small but positive, there was no indication in any of these three cases of spot elongation or separation as seen in the checks on technique. Thus, no electrophoretic evidence for separability of lignin and carbohydrate was obtained.

In another approach, lignin-carbohydrate materials have been subjected by various workers to enzymatic hydrolysis with the partial release of carbohydrate and shifts toward smaller average molecular size in gel filtration curves (16,20,

21). Evidence of this nature supports the implication that the carbohdyrate is bonded to lignin.

In this work on white birch the results of enzymatic reaction and subsequent gel filtration of Fractions 8-11 from the fifth liquor aliquot collected during delignification are shown in Fig. 7. The enzyme used was Onozoka SS, a polysaccharidase including a cellulase, mannanase and xylanase (22). From Fig. 7 it is clear that enzymolysis has caused a shift toward smaller average molecular size without any significant change in the general shape of the curve for UV absorbing material. Thus, by implication, in at least some of the fractions ex-Porasil B the carbohydrate could be bonded to the lignin solubilized by peroxyacetic acid.

[Fig. 7 here]

Changes in optical properties

In earlier work on peroxyacetic acid solubilized lignin from loblolly pine there was a change from positive to negative optical rotation in the fractions ex-Porasil. Somewhat comparable trends have been found in this case for white birch, as illustrated by the specific rotation data in Fig. 8, in which the usual sign convention for the y-axis has been reversed to facilitate comparison with Fig. 6.

[Fig. 8 here]

There is no basis for expecting that specific rotations as in Fig. 8 reflect carbon asymmetry in phenylpropane units of lignin (23). Comparison of Fig. 8 with Fig. 6 makes it apparent that the trends in negative rotation (Fig. 8) essentially follow the trend toward more total polysaccharides being present in the freeze-dried solids, as delignification proceeds (Fig. 6). Superimposed on the latter trend are the trends in polysaccharide composition discussed in connection with Table III and it is noted that increase in the relative amount of xylan as delignification proceeds allows for an increase in xylan polymer chain length.

The relation between oligosaccharide chain length and specific rotation, an additive property, has been reviewed by Géczy (24). For the case of corncob xylan, which has the same 1→4 linked D-xylopyranose structure as wood xylan (25), with increase in the number of xylopyranose units specific rotation becomes more negative (26). Thus, the trends toward most negative rotation (Fig. 8) possibly reflect an increase in xylan chain length as the relative amount of xylan in the polysaccharides (Table III) and the total amount of polysaccharides in the freeze-dried solids (Fig. 6) increases.

Similar reasoning can be applied to the trends toward most positive rotation in Fig. 8. With regress toward the initial stages of delignification there is not only less xylan present in the polysaccharide on a relative (Table III) and absolute basis (Fig. 6) but also relatively more glucan and galactan (Table III). These trends would decrease the contribution from xylan to negative rotation and be in the direction of increasing positive rotation on the basis that the series from cellotriose to cellopentose (27) and galactan from white birch (28) have positive rotations.

The data in Fig. 8 on optical activity (which arises from a difference in speed for right and left circularly polarized light) were all obtained at a wavelength of 546 nm. When changes in optical activity at various wavelengths are measured an optical rotatory dispersion (ORD) curve is obtained and a circular dichroism (CD) curve is obtained in the analogous case for a difference in absorption instead of speed. It is well established that when an asymmetric carbon is attached to a functional group which has an absorption maximum, anomalous ORD and CD curves, or Cotton effects, are observed in that region (29). When a Cotton effect is observed it also means that the absorbing group and asymmetric carbon are relatively close since with separation by one or two carbon atoms the effect is lost (30,31).

From the above, if the carbohydrates responsible for the optical activity seen in Fig. 8 are bonded to lignin so that an asymmetric carbon is attached to or close to the aromatic ring, then Cotton effects could be seen around 280 nm, for example.

On the other hand, some potential types of lignin-carbohydrate linkages (16) such as ester linkages to uronic acid residues would be less likely to allow for observing Cotton effects than an arylglycosidic linkage, for example. Also, the material from the fractions ex-Porasil could be more heterogeneous than homogeneous from a molecular structure viewpoint and the specific rotations in Fig. 8 could reflect trends in populations of molecules which probably have more similarity in hydrodynamic volume than in all specifics of chemical structure. Furthermore, Cotton effects can be either positive or negative (29) so that if a material has molecules with different structures a positive could cancel a negative effect and no Cotton effect could be observed.

As a check on the procedures used in this work a CD curve was plotted from ellipticity data obtained on recrystallized phenyl-β-D-glucopyranoside and found to fit expectations on the basis of the known ORD and UV curves (32). Freeze-dried materials from fractions ex-Porasil, for ORD and CD curves as in Fig. 9, were selected as indicated in Fig. 8 mainly on the basis of observed trends toward most positive (A) and most negative (F) specific rotations.

[Fig. 9 here]

Figure 9 clearly shows Cotton effects occur in the aromatic absorption region for ORD and CD curves and that these effects can be either positive (Fig. 9A) or negative (Fig. 9F). Respectively, these relate to the early and late fractions of freeze-dried materials ex-Porasil near the start (Fig. 9A) and end (Fig. 9F) of delignification. In progressing from the early to late fractions

a positive Cotton effect first becomes less obvious (Fig. 9B and C), then appears to be lost (Fig. 9D), after which a negative Cotton effect appears (Fig. 9F).

Although ORD and CD have been used in the study of naturally occurring polymers such as proteins, polypeptides, and polysaccharides (33) it is believed that this is the first report involving lignin. It is particularly interesting that fractionation ex-Porasil, which is considered to proceed mainly on the basis of hydrodynamic size, has resulted in some fractions having sufficiently specific molecular structures to show the observed Cotton effects. This seems likely to have occurred because hydrodynamic size relates to the composition and specific rotation of the carbohydrate moiety in such a way that there is a significant ordering of molecular structures capable of giving Cotton effects. Furthermore, it is concluded that these Cotton effects arise from the carbohydrates being bonded to the phenylpropane units of lignin.

EXPERIMENTAL

Raw material, delignification and fractionation on Porasil B

The preparation of peroxyacetic acid and wafers from a 30-year-old white birch, the continuous flow reactor used in delignification, and the fractionation on Porasil B were as described previously (1).

When determining liquor concentrations before fractionation some practical difficulty was encountered in obtaining a materials balance on ovendry dissolved solids. A trend to inflated dissolved solids is believed to arise from the presence of 3-hydroxybutyric acid which is probably formed when quenching the oxidant (5).

Methylation

Fractions 1-65 from Aliquots 3-6 (3.6 g) were combined,then added to methanol (180 ml) and ion exchange resin Amberlite IR-120 (4.8 g). After this mixture was

refluxed overnight, the resin was removed, the methanol replaced by water, and the methylated sample fractionated on Porasil B to give results as in Fig. 2.

Analytical procedures

Carboxyl contents, polysaccharide composition and absorptivities were determined as indicated previously (1), and methoxyl was determined according to TAPPI T 209 sw-69.

Spectroscopic measurements

Nuclear magnetic resonance spectra were obtained for samples of freeze-dried materials (80 mg) dissolved in DMSO-d_6 (0.4 ml) using a Varian A60A NMR spectrometer. Location of the hydroxyls was accomplished by noting the disappearance of any of the peaks found in the above spectra and the appearance of an HDO peak after D_2O addition to the above solutions.

Infrared spectra were obtained by transmittance measurements using BDH infrared grade KCl pellets containing exactly 2.00 mg of sample dried over P_2O_5 and 400 mg of dry KCl mixed (1 min) in a stainless-steel capsule on a mixer-mill. Pellets were formed in a conventional press allowing 2 min under vacuum, 3 min under 0.5 ton, and 10 min under 10 tons force. Preliminary and more detailed spectra were obtained on Perkin-Elmer Model No. 700 and 621 infrared spectrometers, respectively, with a pure KCl pellet in the reference beam to compensate for disk absorption and/or scattering. ΔT values were determined by measuring the distance (mm) from the 1535 cm^{-1} minimum absorbance (arbitrarily set between 97 and 98% transmittance) and the 1510 cm^{-1} maximum absorbance.

Paper electrophoresis

Glass fiber chromatography sheets (5 x 6 inches) were presoaked (minimum 10 min) in buffer (0.05N NaOH) prior to use. These sheets were then blotted,

placed in a Gelman electrophoresis chamber (34), and spotted about 1 inch from the anodic side with 30-40 µl of sample solution (1.5% in buffer).

The power (250 V and 40-60 ma) was then turned on for the desired amount of time, after which the sheets were horizontally removed and set aside to dry. Location of the polysaccharides and lignin was accomplished by staining with α-naphthol and p-anisidine solutions (19) and by UV absorbance measurements at 280 nm on the water extracted materials from successive 1-cm strips of the chromatogram, respectively.

Enzymatic hydrolysis

An enzymatic hydrolysis with Onozoka SS and subsequent fractionation of a freeze-dried sample (133 mg) of Fractions 8-11 from Aliquot 5 was accomplished using the procedures of Cheng (22). The enzymatic reaction was monitored until a constant drainage time through a Cannon 50 viscometer was obtained. The enzyme hydrolyzate was then fractionated on a column (52 cm length, 2 cm ID) packed with Sephadex G-75 and the eluate monitored by UV absorbance at 280 nm to give results as in Fig. 7.

Optical measurements

A Perkin-Elmer 141 MC polarimeter equipped with a quartz 1 dm cell maintained at 20°C was used to obtain optical rotations at 546 nm for solutions of freeze-dried materials at known concentrations of between 3-5 g/liter.

A JASCO ORD/CD/UV-5 spectropolarimeter equipped with a 350-watt xenon light source was used to obtain optical rotatory dispersion and circular dichroism curves in the 400 to 200 nm region of representative freeze-dried fractions with known concentrations near 1 g/liter. Several cells in the 10 cm to 1 mm pathlength range, maintained at room temperature, were used to obtain data for the curves as in Fig. 9.

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Table I. Lignin analyses before and after peroxyacetic acid reactions

Determination	Extracted wood wafers	
	Unreacted	Reacted
Yield, %[a]	100	80.9[b]
Klason lignin, %	17.4	2.9
Acid sol. lignin, % (3)	4.8	3.5
Lignin removed, % total lignin	nil	71.2
Apparent material loss, %	nil	3.3
Polysaccharide loss (sugar anal.), %	nil	4.3

[a]Percentages on o.d. extracted wood basis, unless noted otherwise.

[b]Mean of duplicate runs for 4 hr at 51-52°C.

Table II. Peroxyacetic acid reaction time with corresponding liquor aliquots and dissolved solids, as delignification proceeds

Reaction time, hr	Liquor		Delignification, %
	Aliquot, no.	Dissolved solids, % o.d.w.	
0-1.3	1	2.6	6.3[a]
1.3-2.7	2	4.8	23.9[b]
2.7-4.0	3	5.1	45.9[b]
4.0-5.3	4	5.0	63.1[a]
5.3-6.7	5	4.3	77.5[a]
6.7-8.0	6	3.5	84.5[b]

[a]By graphical estimation.

[b]By analysis for Klason and acid soluble lignin.

Table III. Normalized polysaccharide composition of freeze-dried solids

Groups of Fractions	X[a], etc.	Delignification, %					
		6.3	23.9	45.9	63.1	77.5	84.7
12-15	X	31.1	43.3	44.7	68.6	72.9	76.7
	D	25.6	26.9	34.0	8.6	7.6	6.8
	G	23.3	14.9	10.6	10.5	10.6	8.8
	A	13.3	7.5	5.3	7.6	6.5	5.2
	M	1.1	3.0	2.1	1.9	0.6	0.4
	R	5.6	4.5	3.2	2.9	1.8	2.0
28-30	X	42.5	48.7	56.0	64.0	69.1	79.3
	D	34.0	33.7	20.4	20.2	17.3	5.7
	G	14.2	11.2	12.7	6.7	5.4	7.3
	A	5.7	3.7	5.1	6.7	6.5	5.7
	M	2.0	1.9	5.1	1.7	1.1	1.4
	R	1.7	0.7	0.8	0.7	0.6	0.6
44-45	X	42.2	53.6	55.8	69.7	77.5	83.7
	D	32.3	17.9	14.8	7.5	5.8	3.1
	G	14.9	17.9	14.8	9.4	5.8	4.6
	A	7.4	8.9	11.2	9.4	6.9	6.2
	M	2.5	1.3	3.0	3.8	3.5	1.6
	R	0.7	0.4	0.4	0.2	0.5	0.8
52-54	X	45.2	59.1	55.1	76.8	79.0	80.1
	D	27.1	16.9	14.6	3.5	3.0	3.7
	G	13.6	8.4	14.6	5.2	6.0	5.6
	A	9.0	12.7	11.7	10.5	8.0	6.2
	M	4.5	2.5	2.9	3.5	3.0	3.7
	R	0.5	0.4	0.9	0.5	1.0	0.6
58-61	X	40.7	51.9	64.7	61.0	70.5	79.9
	D	26.4	16.5	6.5	9.8	8.9	3.7
	G	14.2	4.7	8.6	8.5	5.5	4.8
	A	10.2	14.2	10.8	11.0	8.2	7.4
	M	8.1	11.8	8.6	8.5	5.5	3.2
	R	0.4	0.9	0.9	1.2	1.4	1.1

[a]Saccharides, expressed as a percentage of the total polysaccharide in the freeze-dried fractions, X-xylan, D-glucan, G-galactan, A-arabinan, M-mannan, and R-rhamnan.

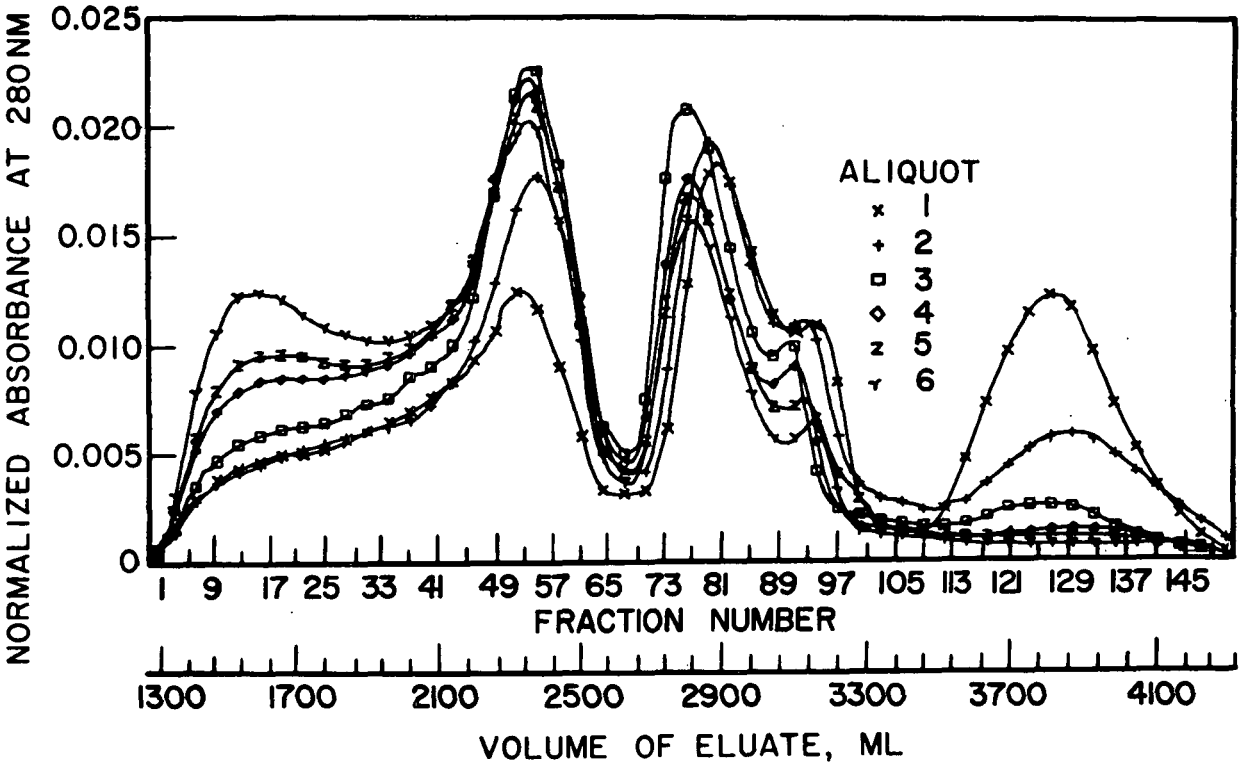

Fig. 1. Normalized absorbance at 280 nm versus fraction number and volume of eluate for Aliquots 1-6.

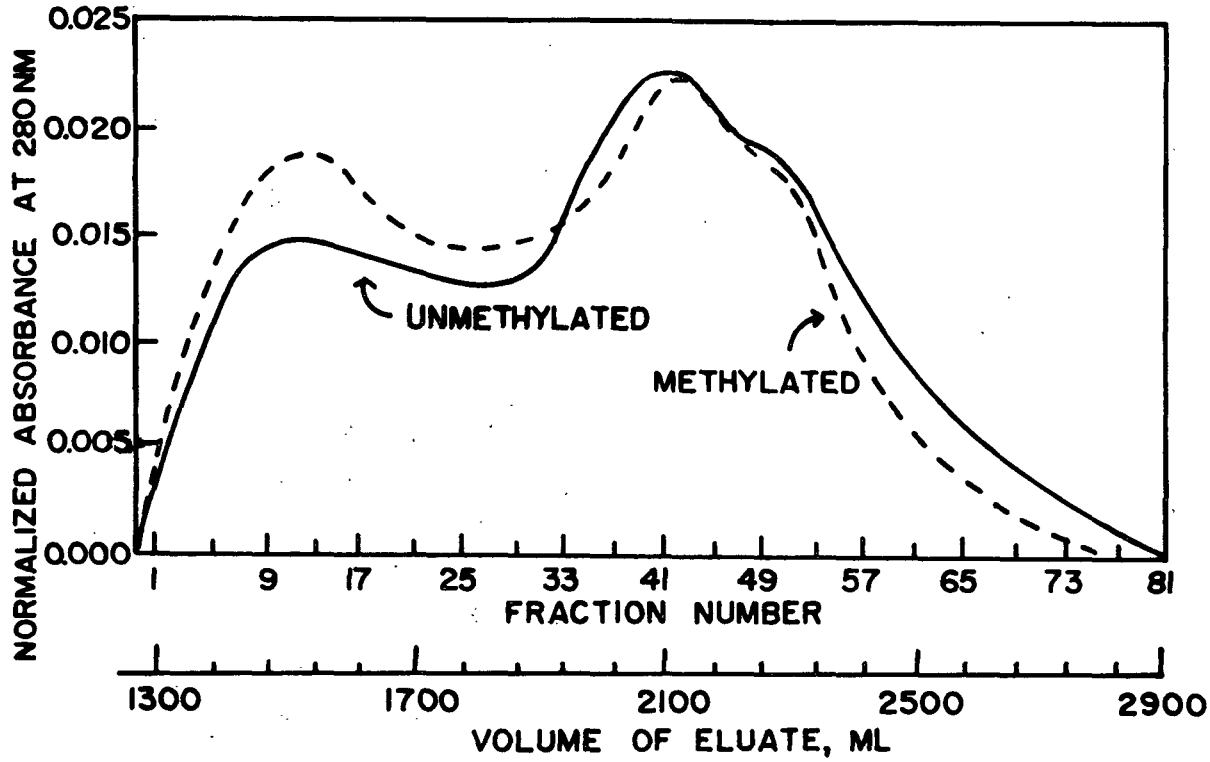

Fig. 2. Normalized absorbance versus fraction number and volume of eluate for combined material (see text) before and after methylation.

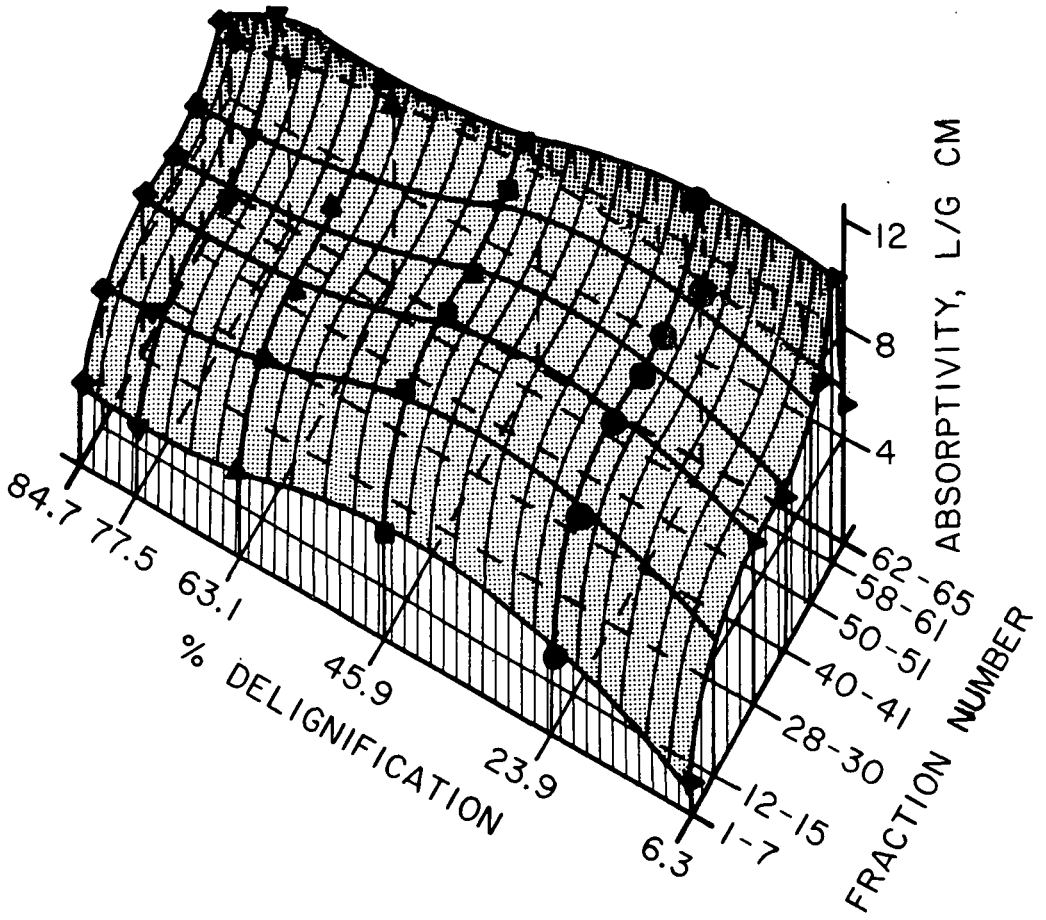

Fig. 3. Variation in absorptivity as delignification and fractionation proceeded.

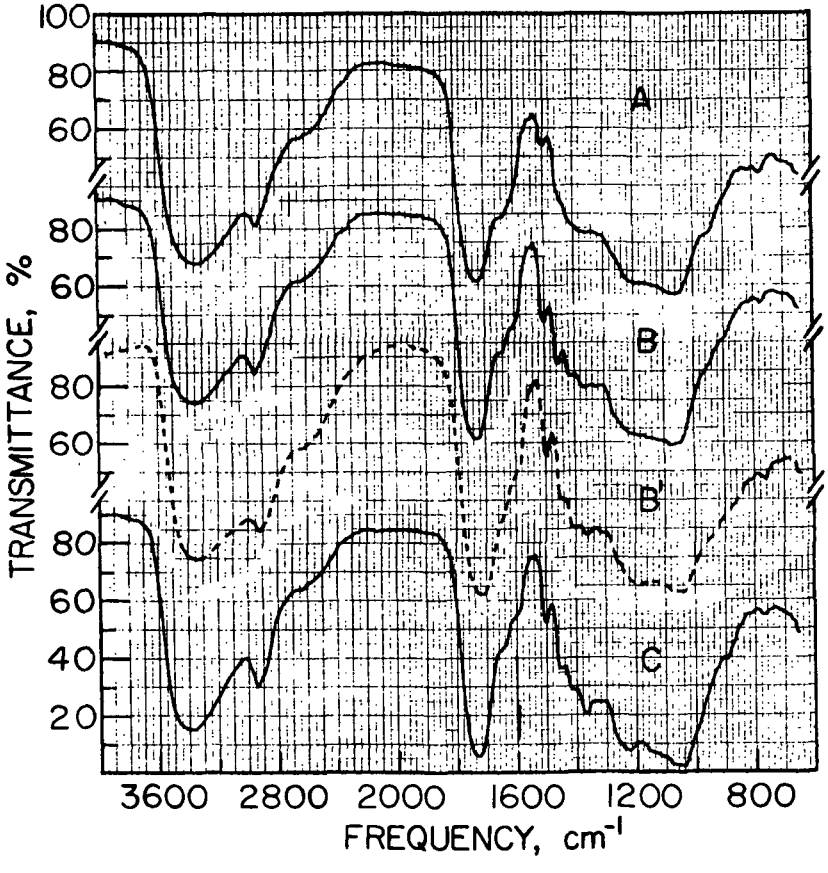

Fig. 4. Infrared spectra of Fractions 12-15 from Aliquots 1, 4, and 6 (Table II) and Fractions 62-65 from Aliquot 4 (curves A, B, C and B', respectively).

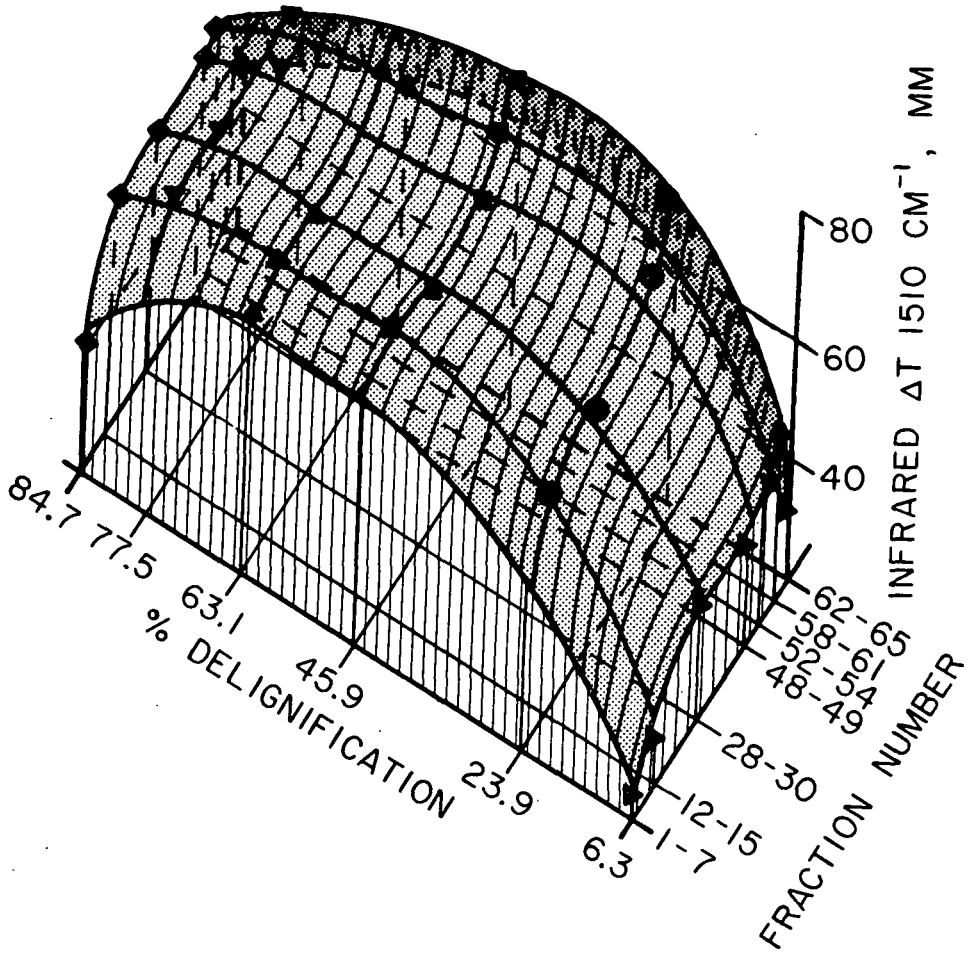

Fig. 5. Variation in infrared $\Delta\underline{T}$ values at 1510 cm^{-1} as delignification and fractionation proceeded.

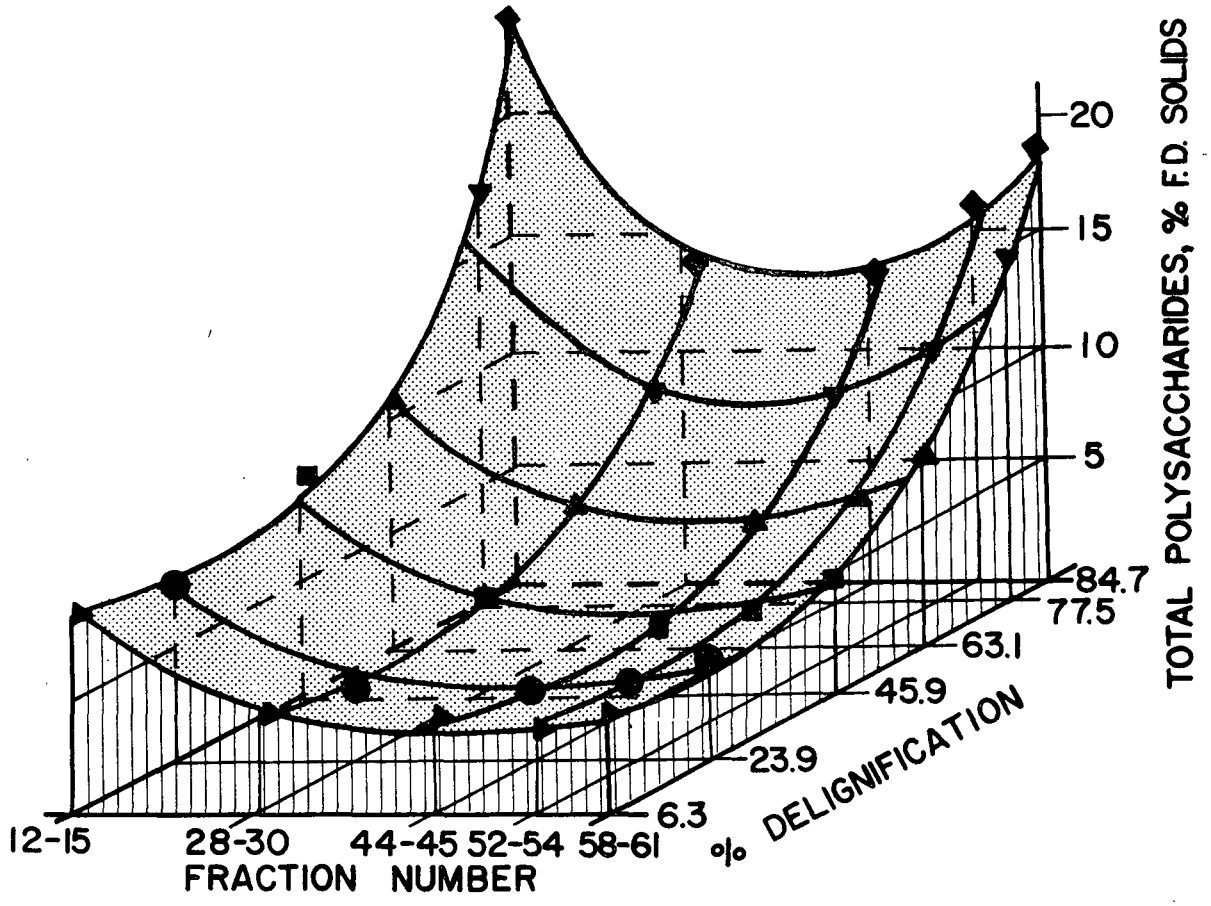

Fig. 6. Variation in total polysaccharides in freeze-dried solids as delignification and fractionation proceeded.

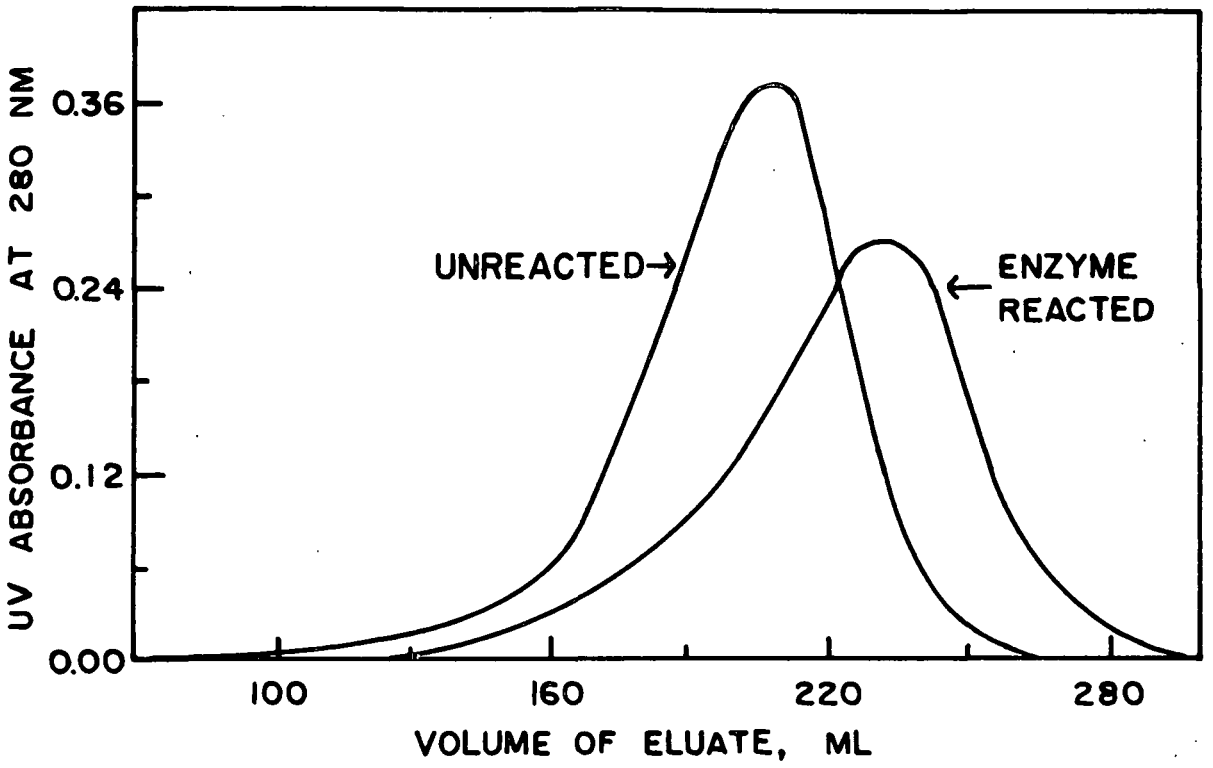

Fig. 7. UV absorbance at 280 nm versus volume of eluate for Fractions 8-11 from Aliquot 5 before and after enzymatic hydrolysis, using a column packed with Sephadex G-75.

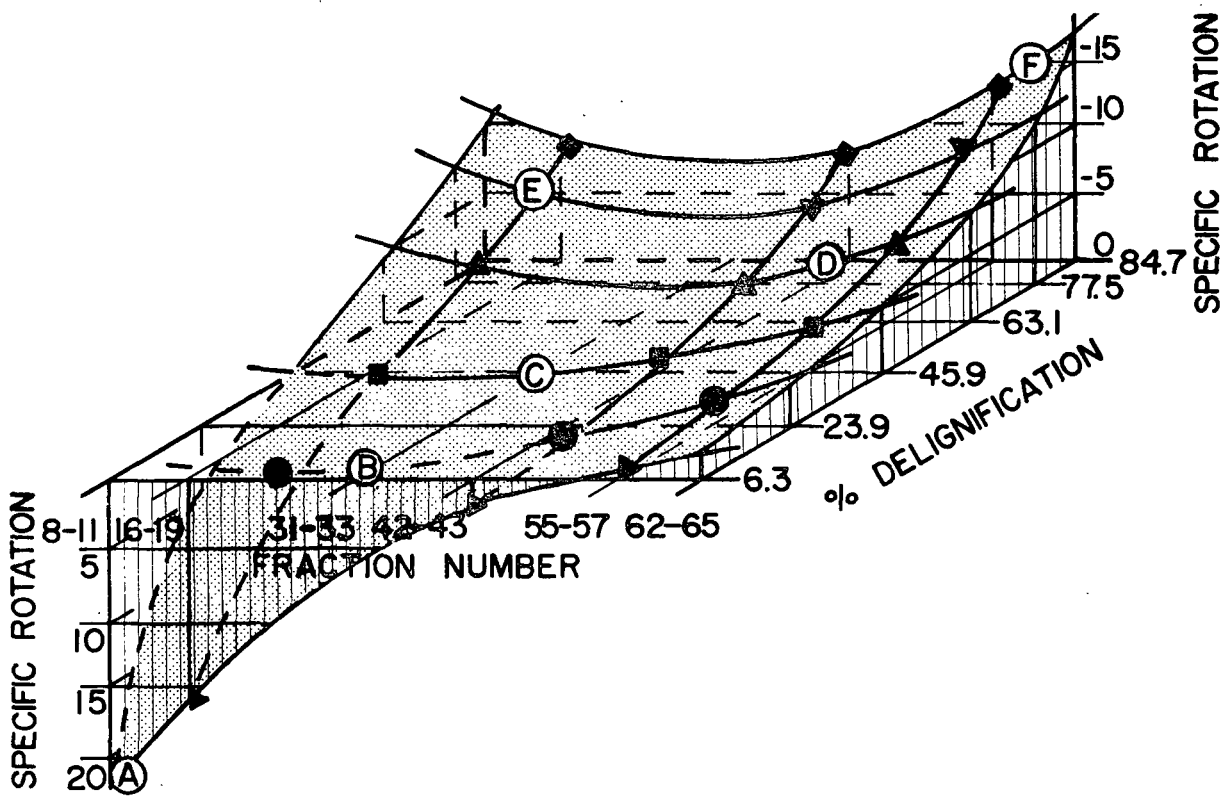

Fig. 8. Variation in specific rotation as delignification and fractionation proceeded. A, B, C, etc., correspond to samples used for curves in Fig. 9.

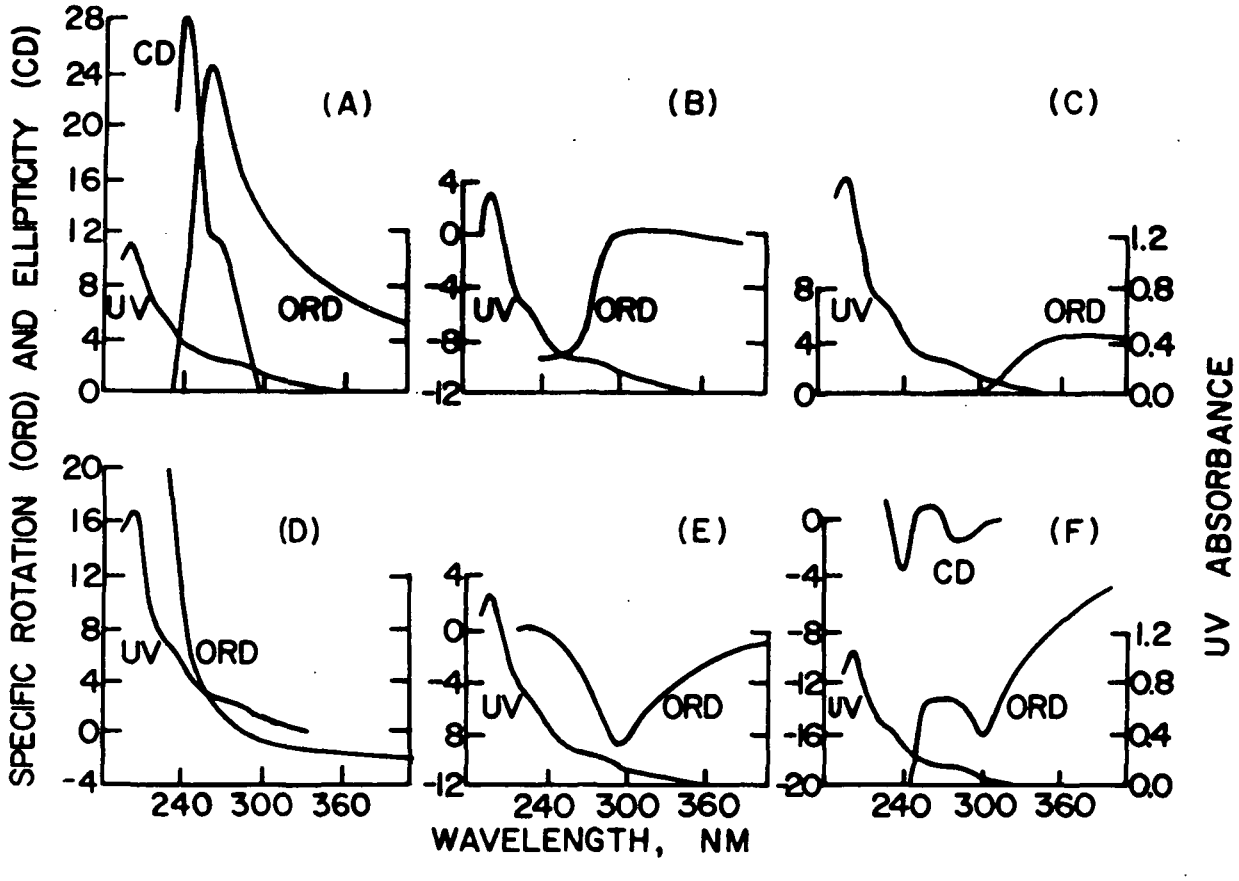

Fig. 9. ORD and UV spectra for Fractions 8-11 (A), 16-19 (B), 24-27 (C), 31-33 (D), 48-49 (E) and 58-61 (F) from Aliquots 1, 5, 2, 3, 4, and 6, respectively. CD spectra are also shown for A and F.